

New England Biolabs Certificate of Analysis

Product Name: *Thermolabile USER[®] II Enzyme*

Catalog Number: *M5508L*

Concentration: *1,000 U/ml*

Unit Definition: *One unit is defined as the amount of enzyme required to nick 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base in 15 minutes at 37°C in a total reaction volume of 10 µL in 1X T4 DNA Ligase Buffer.*

Packaging Lot Number: *10086074*

Expiration Date: *10/2022*

Storage Temperature: *-20°C*

Storage Conditions: *25 mM KCl, 35 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 15 mM Tris-HCl, 100 µg/ml BSA, 50 % Glycerol, (pH 7.5 @ 25°C)*

Specification Version: *PS-M5508S/L v1.0*

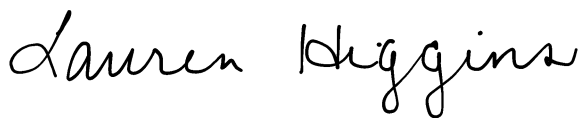
| Thermolabile USER [®] II Enzyme Component List | | | |
|---|--|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| M5508LVIAL | Thermolabile USER [®] II Enzyme | 10086075 | Pass |
| B7204SVIAL | CutSmart [®] Buffer | 10078757 | Pass |

| Assay Name/Specification | Lot # 10086074 |
|---|----------------|
| <p>Functional Testing (Thermolability, Endonuclease III)</p> <p>A 10 µl reaction in CutSmart[®] Buffer containing 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base and 1 unit of Thermolabile USER[®] II Enzyme was incubated for 15 minutes at 37°C followed by heat inactivation for 10 minutes at 65°C. The addition of 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single AP site and incubation for 15 minutes at 37°C followed by 10 minutes at 75°C, results in no cleavage of additional substrate.</p> | Pass |
| <p>Functional Testing (Thermolability, UDG)</p> <p>A 10 µl reaction in CutSmart[®] Buffer containing 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base and 1 unit of Thermolabile USER[®] II Enzyme was incubated for 15 minutes at 37°C followed by heat inactivation for 10 minutes at 65°C. The addition of 10 pmol of a 34 mer fluorescently labeled oligonucleotide duplex containing a single uracil base with 20 units of Endonuclease III and incubation for 15 minutes at 37°C followed by 10 minutes at 75°C, results in no cleavage of additional substrate.</p> | Pass |

| Assay Name/Specification | Lot # 10086074 |
|--|----------------|
| <p>Functional Testing (USER, Transformation assay) A 10 µl reaction in ThermoPol® Reaction Buffer containing 20 ng linearized pNEB206A, 100 ng of a 950 bp control PCR product and 1 unit of Thermolabile USER® II Enzyme was incubated for 15 minutes at 37°C followed by 15 minutes at 25°C. After transformation into ER2267 chemically-competent cells >95% of colonies contained recombinant plasmid.</p> | Pass |
| <p>qPCR DNA Contamination (E. coli Genomic) A minimum of 1 unit of Thermolabile USER® II Enzyme is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.</p> | Pass |
| <p>RNase Activity (Extended Digestion) A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of Thermolabile USER® II Enzyme is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p> | Pass |

This product has been tested and shown to be in compliance with all specifications.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.



Lauren Higgins
Production Scientist
02 Oct 2020



Michael Tonello
Packaging Quality Control Inspector
02 Oct 2020